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Award Number: DAMD17-99-1-9505

TITLE: Chemoprevention of Ovarian Cancer

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REPORT DATE: October 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
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20050408 042

**REPORT DOCUMENTATION PAGE**Form Approved  
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

**1. AGENCY USE ONLY**  
(Leave blank)**2. REPORT DATE**  
October 2004**3. REPORT TYPE AND DATES COVERED**  
Annual (1 Oct 2003 - 30 Sep 2004)**4. TITLE AND SUBTITLE**

Chemoprevention of Ovarian Cancer

**5. FUNDING NUMBERS**

DAMD17-99-1-9505

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REPORT NUMBER****9. SPONSORING / MONITORING  
AGENCY NAME(S) AND ADDRESS(ES)**U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012**10. SPONSORING / MONITORING  
AGENCY REPORT NUMBER****11. SUPPLEMENTARY NOTES****12a. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

**12b. DISTRIBUTION CODE****13. ABSTRACT (Maximum 200 Words)**

The overarching hypothesis of this program project is that 4-HPR (a synthetic vitamin A) and oral contraceptives (OCP) induce apoptosis, possibly through induction of TGF $\beta$  production by stromal cells, as well as by direct interaction with the surface epithelial cells, and these two cell types may act synergistically. In Project 1, 18 adult Rhesus monkeys were give 4-HPR, OCP, the combination, or no medication for 3 months. There were consistent differences in the absolute fluorescence intensities and relative contributions noted between pre- and post-drug measurements in each drug group. A second study involving 30 Cynomolgus macaques and using a crossover design has been completed; immunohistochemical analysis of several biomarkers and analysis of the fluorescence spectroscopy data are ongoing. Project 2 has been transferred to the University of Arizona with the relocation of Dr. Molly Brewer. Now that a supply of 4-HPR has been obtained from the NCI, this study should be ready for patient accrual within the next few weeks pending final approval of revisions by HSRRB. In Project 3, we have focused on understanding the mechanism of action of 4-HPR in tissue culture using both normal and immortalized epithelial cells. Studies are ongoing, and results to date are inconclusive.

**14. SUBJECT TERMS**

Ovarian cancer

**15. NUMBER OF PAGES**

27

**16. PRICE CODE****17. SECURITY CLASSIFICATION  
OF REPORT**

Unclassified

**18. SECURITY CLASSIFICATION  
OF THIS PAGE**

Unclassified

**19. SECURITY CLASSIFICATION  
OF ABSTRACT**

Unclassified

**20. LIMITATION OF ABSTRACT**

Unlimited

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## INTRODUCTION

Ovarian cancer is the second most common malignancy of the female genital tract in the United States. No effective screening tool exists. Consequently, over 70% of cases are diagnosed after the cancer has already spread beyond the ovary. For women with stage III epithelial ovarian cancer—the most common stage—the 5-year survival is no higher than 20%. Clearly, early detection and prevention of this disease are critical issues. The overall goals of this program project are: 1) to develop innovative strategies for prevention of ovarian cancer through the assessment of the potential effect of oral contraceptives (OCP) and retinoids (Vitamin A derivatives) on the ovary and identification of molecular markers and mechanisms associated with the chemopreventive activity of these compounds, and 2) to assemble a multidisciplinary team that will become a world leader in the field of ovarian cancer prevention. A large body of epidemiologic evidence supports the fact that OCP can reduce a woman's risk of ovarian cancer as much as 50%. Similarly, preliminary data from a large Italian randomized chemoprevention trial for secondary breast cancers suggests that retinoids may have preventive activity against ovarian cancer. In addition, retinoids have been shown to induce apoptosis in normal ovarian surface epithelial cells in the laboratory. The major overarching hypothesis of this program project is that 4-HPR and OCP induce both growth inhibition and apoptosis. Data on 4-HPR suggests this activity is mitochondrial mediated which can be assessed using fluorescence spectroscopy which is sensitive to changes in NADH and FAD, both electron carriers active in the mitochondria. Changes in mitochondrial permeability are thought to be one of the

changes in 4-HPR. Evaluating gene expression is another way of understanding the action of these molecules and the combination of these results should help us discern better methods of prevention of ovarian cancer.

## **BODY**

### **PROJECT 1**

#### **Chemoprevention of Ovarian Cancer Using a Rhesus Primate Model**

The second study, using 30 cynomolgus monkeys in a cross over design has been completed and is being analyzed; the increased numbers of monkeys should overcome the limitations of the numbers in the first study. This protocol was approved by the Animal Care Use Committee at The University of Texas M.D. Anderson Cancer Center and was conducted at the Department of Veterinary Sciences in Bastrop, Texas, where all animals were caged separately. The animals were given 4-HPR (4 monkeys), OCP (5 monkeys), the combination of 4-HPR+OCP (5 monkeys), or no medication (4 monkeys) daily for 3 months. Doses of 4-HPR and OCP were calculated by allometric scaling (1). The OCP used was Ortho-Novum 1/35, a medium-dose oral contraceptive with 1 mg norethindrone and 35 ug ethinyl estradiol in each pill. The 4-HPR dose was calculated in the same manner from the accepted human dose of 200 mg daily. Prior to starting medication and following 90 days of medication, monkeys underwent laparotomy, spectroscopy, and ovarian biopsies. Following the first 3 months of treatment, the group receiving 4-HPR crossed over to the OCP arm, the monkeys receiving OCP crossed over to the 4-HPR arm and the control monkeys crossed over to the combination group. There was a 1 month washout period between crossovers. Following completion of the initial portion of this study, the 9 monkeys that were

negative on Herpes (Monkey B) serology were continued on OCP. A study by Gus Rodriquez (2) has shown that monkeys receiving OCP and progestin for 2 years showed a 5 fold increase in the rate of apoptosis of the ovarian surface epithelial cells over the controls suggesting that the chemopreventive activity of the OCP may be induction of apoptosis of the surface epithelial cells. However, this rate of apoptosis was not observed in our study. We have continued the OCP, changing to the Tri-phasil which was used in the Rodriquez study hypothesizing that the increase in progestin in the third week may have caused the increased apoptosis. Monkeys have undergone ovarian biopsy every 6 months to assess the cumulative response to the OCP. This study was completed in December 2002 and 2 manuscripts are being prepared for publication.

Immunohistochemical markers have been analyzed that suggest that the combination of the OCP and 4HPR have more activity than either alone. ER $\alpha$  was negative as has been reported in other papers. However, ER $\beta$  was positive in the OCP and 4-HPR groups but strongly positive in the combination group suggesting that these 2 drugs have synergistic activity. We saw the same synergism in the results of our fluorescence spectroscopy (3) in the combination group. These findings suggest that these drugs may have activity via activation of the ER $\beta$  receptor as well as an effect on mitochondrial metabolism. RAR  $\alpha$ ,  $\beta$ ,  $\gamma$  evaluation suggests that RAR  $\alpha$  and  $\gamma$  are upregulated with the combination but ER $\beta$  is not. There has been much work, particularly in cell lines, evaluating the effect of retinoids on the RAR's. 4-HPR has been suggested to be receptor independent but induce apoptosis and growth inhibition through changes in the mitochondria. However, this data from monkeys suggests that

some of the activity of this drug may be mediated through the nuclear receptors. Interestingly, OCP seems to increase this activity, again suggesting a synergistic response between the 2 drugs. This paper is also being prepared for publication.

## **PROJECT 2**

### **Chemoprevention of Ovarian Cancer: Modulation of Biomarkers in Women at Low- and High-Risk for Ovarian Cancer Using Fenretinide (4-HPR) and Oral Contraceptives**

The clinical trial in Project 2 has been activated at the University of Arizona following approval by both the University of Arizona IRB and the Human Subjects Protection at the Department of Defense. We have received the OCP from Ortho-McNeil and the 4-HPR from the NCI. The FDA has approved release of the drug with an investigator approved IND. The protocol was modified by the FDA and is currently being re-reviewed by the DOD. We anticipate this trial will have recruited patients within the next 6 weeks. We have applied for and been approved for a no-cost extension due to the extreme difficulty in gaining the FDA and IRB approvals needed to start this project.



## **PROJECT 3**

### **Chemoprevention of Ovarian Cancer: Molecular Mechanisms and Markers Laboratory Investigations of 4-HPR and OCP**

We investigated the effects of 10 $\mu$ M 4-HPR on growth of the IOSE29 series of transformed cell lines (IOSE29 with SV40; IOSE29 with SV40 and telomerase; IOSE29 with SV40, telomerase, and H-ras; IOSE29 with SV40, telomerase, and K-ras) under normal growth conditions (1:1 MCDB 105 and Medium 199; 10%FBS) 3 and 5 days after treatment. We used NOE119 cells and OVCA433 cells as benchmarks by which to gauge the responses by the transformed cell lines. After 3 days, there was no notable difference in growth inhibition between the NOE119 cells and the OVCA433 cells, but the transformed cell lines saw greater than 50% inhibition when compared to the control group treated with DMSO alone. At 5 days post treatment, when the differential growth inhibition between NOE119 and OVCA433 is substantial, the transformed cell lines exhibited similar growth inhibition to that of OVCA433. We will be conducting the same experiment with the IOSE80 series of transformed cells in the near future.

We also investigated the effects of 1, 2, 5, and 10  $\mu$ M ShetA2 under normal growth conditions (1:1 MCDB 105 and Medium 199; 15% FBS) for 5 days on 9 normal ovarian epithelial cell lines (NOE110L, NOE113L, NOE116L, NOE117R, NOE118L, NOE119L, NOE072, NOE097, NOE099) and 6 different ovarian cancer cell lines (OVCA420,

SKOV3, DOV13, OVCA432, OVCA429, OVCA433). We found that at concentrations of 5 $\mu$ M and 10 $\mu$ M, ShetA2 was equally as toxic to NOE cells as it was to OVCA cells. It does not appear to have differential growth inhibition effects between normal cells and cancer cells.

Since we found differential growth inhibition 5 days post-treatment of 10 $\mu$ M 4-HPR under normal growth conditions between normal ovarian epithelial cells and ovarian cancer cells, we are planning further experiments. We will look focus on 2 ovarian cancer cell lines (OVCA433 and OVCA429) and 2 normal ovarian epithelial cell lines (NOE113L and NOE119L). Our plan is to grow the cells simultaneously for apoptosis, cell cycle, and microarray assays for 1, 3, and 5 day time points after treatment of 10  $\mu$ M 4-HPR. We are trying to determine whether 4-HPR is causing apoptosis or cell cycle arrest. The microarray study will help pinpoint genes associated with the mechanism behind 4-HPR.

Work in progress and in the planning stages include studies that focus on determining the effects of 4-HPR and TGF $\beta$  in inducing apoptosis and quantifying signaling in NOE cells treated with TGF $\beta$ , 4-HPR and OCP components. Our initial examination of induction of apoptosis in OVCA420, NOE 115 and NOE 106 treated cells have been inconclusive and currently are in the process of being repeated using the Tunel Assay (Apoptag®).

## **Administrative Core**

During this reporting period, there have been no changes in key personnel. Dr. David Gershenson, PI, continues to meet with the administrative assistant on a weekly basis. The administrative assistant coordinates and schedules all grant-related meetings and conference calls, facilitating interactions and communications between investigators. Conference calls are conducted once a month to 1) review research activities and discuss scientific issues related to grant activities and 2) identify any problems or barriers associated with research and to assure that all goals are being met within realistic time and budget constraints.

Financial accounts have been established for the University of Arizona's subcontract with Dr. Molly Brewer and all projects and cores at The University of Texas M. D. Anderson Cancer Center. The administrative assistant monitors each account on a monthly basis to ensure that there are no problems or discrepancies.

The administrative assistant is also responsible for timely submission of all reports to the Department of Defense.

## **Histopathology Core**

The purpose of the Histopathology Core is to provide central and uniform histopathologic, immunohistochemical, in-situ hybridization, and apoptosis assay support to the projects in this grant. Histopathologic evaluation, immunohistochemistry, in-situ hybridization, and evaluation of apoptosis have a central role in the design of these projects. The Histopathology Core, in using one central lab for this purpose, will promote uniformity of results by controlling variables associated with specimen handling, and with the technical performance and interpretations of these tests.

For Project 1, biopsies from 18 Rhesus monkeys before treatment and after treatment (for a total of 53 specimens) were fixed, processed, and embedded in paraffin blocks. These blocks were sectioned and stained with hematoxylin and eosin for histologic evaluation. A pathologist associated with the core (M.D.) reviewed these H&E slides. Immunohistochemical staining was then performed on all of the specimens. The markers included BAX, BCL-X, EGFR, ER, Her-2, Ki-67, p21, p53, PR, TGF beta, TGF beta-RI, TGF beta-RII. The 636 immunohistochemical stains were then evaluated and quantified. These results were then given to the investigator. Next the 53 specimens were evaluated for apoptosis using APO-TAG. These assays were reviewed and evaluated with the results forwarded to the investigator. In-situ hybridization for RAR-beta on these specimens is pending. An additional 30 primates have undergone three

surgeries each with two biopsies being obtained at each surgery (180 specimens). These specimens have been processed and the immunohistochemical stains have been partially completed.

As Project 2 is not yet underway, no specimens have been processed by the Histopathology Core.

In support of the Idea Grant, immunohistochemical staining was reviewed and evaluated on seven cell lines. These cell lines included NOE 71, 72, 78, 79, 80, 83, and 86. Cytospins and smears from each of these lines were stained with AE1/AE3 and vimentin immunohistochemistry. The stains were reviewed and evaluated with the results forwarded to the investigator.

Please refer to the individual project summaries for the incorporation of the results of these assays and their implications.

## **CONCLUSIONS**

### **Project 1:**

The Statement of Work for this project has been completed. However, we have completed a second study involving 30 cynomolgus monkeys in a crossover study design. The results of this study will be submitted within the next month for publication.

### **Project 2:**

Last year, this project was transferred to the University of Arizona, where Dr. Molly Brewer relocated. The protocol was subsequently approved by both the University of Arizona IRB and the Human Subjects Protection at the Department of Defense. The 4-HPR supply has recently been received from the National Cancer Institute. Because of regulatory issues that required clarification, there was a rather lengthy delay in moving forward with final approval. However, the study has been approved by both the University of Arizona IRB and the HSSR. We are recruiting patients to the study now.

## REPORTABLE OUTCOMES

Brewer M, Johnson K, Follen M, Gershenson D, Bast R. Prevention of ovarian cancer: intraepithelial neoplasia. *Clin Cancer Res* 9:20-30, 2003.

Brewer M, Wharton JT, Wang J, Gershenson D, Bast R, Zou C. *In vitro* model of normal, immortalized ovarian surface epithelial and ovarian cancer cells for chemoprevention of ovarian cancer. Submitted to *Gynecol Oncol*, 2004.

## APPENDIX

Prevention of Ovarian Cancer: Intraepithelial Neoplasia  
Clinical Cancer Research 9:20-30



## Minireview

# Prevention of Ovarian Cancer: Intraepithelial Neoplasia

Molly A. Brewer,<sup>1</sup> Karen Johnson,  
Michele Follen, David Gershenson, and  
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## Abstract

To reduce the incidence and mortality associated with invasive cancers, the Intraepithelial Neoplasia (IEN) Task Force recommends that carcinogenesis be viewed as a disease that requires treatment. This publication outlines the current knowledge of IEN of the ovary and reviews chemoprevention possibilities for ovarian cancer. Ovarian cancer has the highest mortality of all of the gynecological cancers and is the fourth leading cause of death from cancer in women. The IEN Task Force has defined precancer as a noninvasive lesion that has genetic abnormalities, loss of cellular control functions, and some phenotypic characteristics of invasive cancer with a substantial likelihood of developing invasive cancer. The IEN Task Force recommends targeting moderate to severe dysplasia for new IEN treatment agents in clinical trials. Ovarian cancer does not have a clear preinvasive lesion yet merits considerable study for new prevention strategies because of the high mortality associated with ovarian cancer. There is a great unmet clinical need for treatments that can prevent ovarian cancer by providing nonsurgical options that treat the entire epithelial layer. New prevention strategies hold significant promise to reduce the mortality from ovarian cancer.

## Introduction

Carcinogenesis must be viewed as a disease and disequilibrium that requires treatment to dramatically reduce the incidence and mortality associated with invasive cancers. The IEN<sup>2</sup>

Task Force has defined precancer as a noninvasive lesion that has genetic abnormalities, loss of cellular control functions, some phenotypic characteristics of invasive cancer, and predicts a substantial likelihood of developing invasive cancer. In demonstrating effectiveness and patient benefit of new IEN treatment agents in clinical trials, the IEN Task Force recommends targeting moderate to severe dysplasia, which is close in stage of progression to invasive cancer and thus substantially elevates the risk of developing cancer.

Epithelial ovarian cancer has the highest mortality rate of any of the gynecological cancers; the 5-year survival is no more than 30% despite aggressive treatment. Seventy percent of these cancers are diagnosed with widespread intra-abdominal disease or distant metastases, which partially accounts for the poor prognosis associated with ovarian cancer. Although up to 90% of stage IA tumors and 70% of stage II tumors can be cured by current management, the cure rate drops below 30% for Stage III and IV tumors. Even ovarian cancer limited to the pelvis has a 5-year survival of only 50% (1). This dismal overall prognosis for women with ovarian cancer results from an inability to detect ovarian cancers at an early, curable stage, from the lack of effective therapy for advanced disease, and from our incomplete understanding of both the early changes in the ovary that predate the development of cancer and the initiators of these changes. Although radical surgery, radiation therapy where appropriate, and new methods of chemotherapy have improved survival times, cure rates have stayed essentially the same over the last 20 years. Thus, early intervention with chemopreventive agents merits serious consideration as a desirable alternative to suboptimal treatment of invasive disease.

Cancer chemoprevention is a rapidly growing area of research because of the possibility to prevent disease and to restore cancer-suppressing cellular functions. Chemopreventives are micronutrients or medications that prevent or delay cancer in at-risk populations. Fundamental elements for chemoprevention studies include (a) a suitable cohort of patients with sufficient incidence to establish an acceptable risk:benefit ratio; (b) appropriate agents that are safe and whose use is supported by both epidemiological and mechanistic data; and (c) measurable biomarkers that are likely to be affected by the agent and whose modulation is predictive of the postulated chemopreventive activity (2). Biomarkers are important because they can be used in lieu of following patients prospectively until a cancer occurs, if they indicate a protective response to a chemopreventive agent. Several criteria must be met for biomarkers to be useful: (a) they are relevant to the development of neoplasia either phenotypically (proliferation, angiogenesis, or nuclear morphometry) or mechanistically (molecular markers); (b) they

Received 4/22/02; revised 8/6/02; accepted 8/8/02.

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<sup>2</sup> The abbreviations used are: IEN, intraepithelial neoplasia; OSE, ovarian surface epithelial; EGFR, epidermal growth factor receptor; TGF,

transforming growth factor; 4-HPR, (4-hydroxyphenyl) retinamide; TKI, tyrosine kinase inhibitor; COX, cyclooxygenase; PI3K, phosphatidylinositol 3'-kinase; OCP, oral contraceptive; RA, retinoic acid; RAR, RA receptor; KGF, keratinocyte growth factor; NOE, normal ovarian epithelial; HGF, hepatocyte growth factor.

are modulated by chemopreventive agent; and (c) they should predict a decrease in carcinogenesis (3).

## Epidemiology

**High Epidemiological Risk.** The etiology of ovarian cancer remains unknown; low prevalence rates, low participation rates, small sample sizes, and potential bias in the selection of control groups have limited the interpretation of results from epidemiological studies. However, multiple epidemiological studies agree that an increased risk of epithelial ovarian cancer has been linked to advancing age, family history of breast or ovarian cancer, and frequency of ovulation (4–15). Reproductive factors have been extensively studied, but interpreting these results has been complicated by the intercorrelation of reproductive characteristics (4–15). Despite these limitations, several factors related to ovulation have been consistently associated with increased or decreased risk of developing ovarian cancer. Risk is increased with uninterrupted ovulation (nulliparity), larger number of lifetime ovulatory cycles (early age at menarche and late age at menopause), and possibly hyperovulation (fertility drugs), whereas risk is reduced by factors that suppress ovulation [pregnancy, breast feeding, and OCP use (4–15)].

## High-Risk Population

**High Genetic Risk.** Premenopausal women with a family history of breast and/or ovarian cancer constitute an important high-risk group and are excellent candidates for prevention strategies. Although only 10% of ovarian cancers are attributable to germ-line mutations, this high-risk population is an ideal patient population to target for preliminary chemoprevention studies because of the higher prevalence of ovarian cancer as compared with that seen in the general population. Women from a cohort of high-risk families carrying the BRCA1 mutation were observed to have an approximately 40–60% risk of developing ovarian cancer and an 85% chance of developing breast cancer (15). Multiple methods of calculating risk are available. The Parmigiani method (16) uses Bayes theorem and calculates likelihood ratios. The probability of a mutation in the general population is 0.04%–0.20%, with part of the variation attributable to the ethnic mix of the population. A family history of breast and/or ovarian cancer determines the risk calculation in a particular patient. High-risk family histories with an elevated risk of developing ovarian cancer include >2 breast cancers and 1 or more cases of ovarian cancer at any age, >3 cases of breast cancer before age 50 years, sister pairs with cancers less than age 50 years, cases of breast cancer occurring at or before age 40 years, Ashkenazi Jewish descent (which carries a 2% or greater risk of mutation; Ref. 17), or 1 or more cases of breast/ovarian cancer.

The ideal design for a chemoprevention trial includes a high-risk population with an identifiable and easily accessible preinvasive lesion (e.g., IEN), a safe and effective chemopreventive agent, and surrogate end point biomarkers that have been validated as markers of regression of such lesions. For ovarian cancer chemoprevention trials, the targeted population should include high-risk women with a strong family history of breast/ovarian cancer, with or without a BRCA mutation, or with Ashkenazi Jewish descent. Although there is as yet no

identifiable preinvasive lesion of ovarian cancer (18), there is strong evidence for one based on the increased numbers of inclusion cysts and areas of proliferation noted in the ovaries of high-risk women seen in some studies (19); thus, this has the potential for use as a biomarker.

## Pathology

**IEN in the Ovary.** We hypothesize that there is an IEN precursor to ovarian cancer; however, the natural history of ovarian cancer and the location of the ovary have made it difficult to characterize precursor lesions. The ovary is not routinely biopsied because of the inaccessibility of the ovary in its i.p. location and concern about the effect on fertility that might result from biopsy. Scully (20) has qualitatively described early histological changes in the ovary, whereas Deligdisch *et al.* (21, 22) have described them quantitatively with the use of nuclear texture analysis. These studies support the concept that ovarian cancer behaves in a similar manner to other epithelial cancers with an identifiable precursor. Other authors (23, 24) have described a pathological process in the ovary consistent with the IEN seen in other organ systems that occurred adjacent to existing cancers. Although progression from IEN to cancer has not yet been validated, there is accumulating evidence including a chain of underlying molecular events that supports the ovarian IEN concept.

The cell of origin of epithelial ovarian cancer remains controversial, although most investigators think it is the OSE cell. Many ovarian cancers are thought to arise from OSE cell-lined inclusion cysts (see Fig. 1); these small, subsurface cysts are hypothesized to arise from involution of ovarian surface epithelium at ovulation (20), but some inclusion cysts are thought to antecede ovulation because they are often present in fetal and juvenile ovaries (25). Small collections of malignant cells contiguous with normal ovarian epithelium suggestive of an IEN but not involving underlying tissues can be found in: (a) ovaries removed from women who eventually develop primary peritoneal carcinomatosis (19); (b) high-risk women who undergo prophylactic oophorectomy (19), particularly those with the BRCA1 mutations (24); and (c) in areas adjacent to stage I cancers that show a transition from malignant to normal epithelium (23, 24). However, a characteristic histological precursor lesion for ovarian cancer is not apparent in all prophylactic oophorectomy specimens (26–29). Whether these contradictory findings are due to differences in patient populations or differences in pathological techniques is not clear. However, these findings underscore the discrepancies present in our understanding of IEN of the ovary and suggest that the ovarian surface epithelium is probably the precursor for most epithelial ovarian cancers. OSE cells differ from peritoneal mesothelial cells because they overlie the ovarian stroma and are in close contact with the hormones secreted by the ovary. The chronic repair after ovulation and/or the influence of ovarian hormones are thought to increase the propensity of the OSE cells to undergo tumorigenesis and may account for the higher incidence of ovarian cancer compared with primary peritoneal cancer.

The molecular mechanisms leading to the initiation and progression of ovarian cancer remain elusive, partly because of the ovary's location and the consequent difficulty in identifying

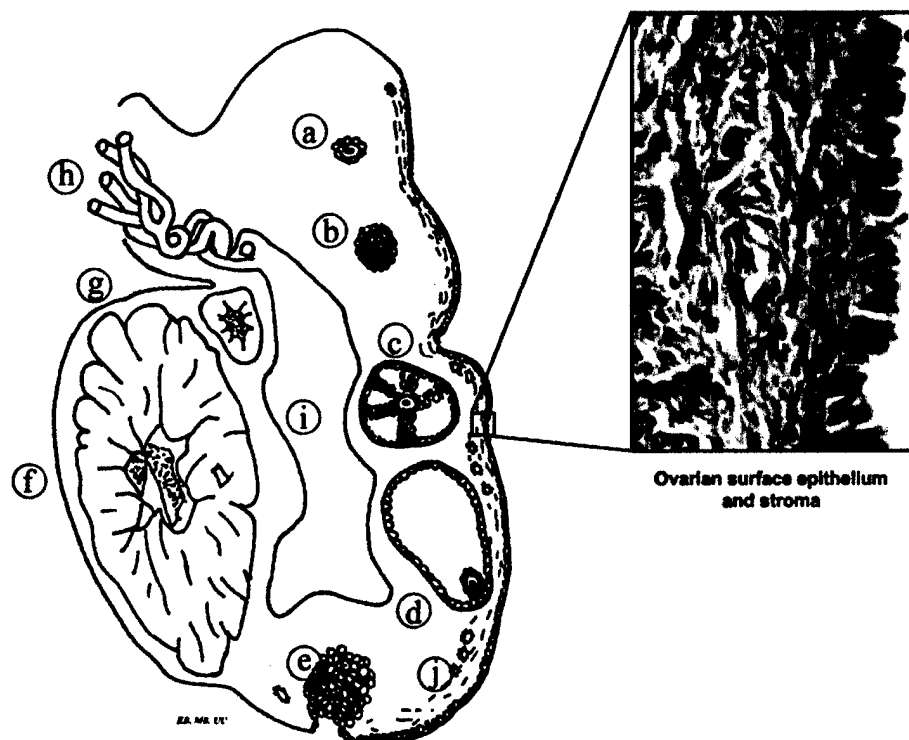


Fig. 1 Normal ovarian structures. a, primary follicle; b, secondary follicle; c, tertiary follicle; d, ovulatory follicle; e, hemorrhagic cyst; f, corpus luteum; g, corpus albicans; h, ovarian artery and vein; i, medulla; j, inclusion cyst.

early or precursor lesions. In contrast, analysis of colon, cervix, and head and neck cancers have resulted in a rapidly emerging understanding of the genetic events underlying the initiation and progression of these diseases and of the biological events that result from these genetic changes.

As with all cancers, ovarian cancer is a consequence of either germ-line or acquired somatic changes in genetic function. The acquired changes in gene expression or function can result from mutations or epigenetic alterations such as changes in methylation. Therefore, one important challenge is to link the genotypic changes that occur in ovarian cancer cells to the phenotypic and biological changes observed in human tumors and cell lines.

Although the association between ovulation and ovarian cancer is well accepted, little is known about the underlying biological mechanisms of this association. Ovulation is thought to be important for the development of inclusion cysts from which ovarian cancers may arise. In addition, ovulation, and particularly the high estrogen associated with ovulation, may provide a stimulus for proliferation of ovarian surface epithelium. Ovaries removed prophylactically from women with a strong family history of ovarian cancer demonstrate increased frequency of occult carcinomas, epithelial hyperplasia and atypia, and increased stromal activity (30). Epithelial hyperplasia and increased number of crypts (which are deep indentations of the ovary covered with surface epithelial cells) with an associated increase in proliferation may contribute to tumorigenesis by increasing the risk that a genetic alteration will occur.

OSE cells are generally quiescent but proliferate after ovulation to repair the defect created by the release of an oocyte from a mature follicle. Increased proliferation may contribute to the accumulation of genetic defects in the OSE cells. Furthermore, growth factors produced during wound healing may promote the survival of OSE cells with accumulated mutations. Alternatively, ovulation may be important for the development of inclusion cysts from which ovarian cancers may arise. Entrapment of ovarian epithelium in the stroma of the ovary may disrupt the normal relationship between the ovarian surface epithelium and the underlying stroma. Disruption of normal epithelial stromal interactions can increase mutation rates, directly contributing to ovarian cancer development. Furthermore, growth factors normally produced by the ovarian epithelium that would diffuse into the large potential space of the peritoneal cavity may be present at higher levels in the microenvironment of entrapped ovarian epithelium. Finally, ovarian epithelium in the stroma may be exposed to higher concentrations of paracrine growth factors produced by the stroma or to higher hormone levels than are present on the surface of the ovary. In support of the concept of increased ovulation conferring increased risk are the pathobiological data that show a strong correlation between the lifetime number of ovulations and the frequency of p53 mutations (19, 30). One biological hypothesis is that ovulation may result in genomic instability that occurs as a result of the repeated turnover of cells that renders cells sensitive to the high levels of gonadotropins or gonadotropin-releasing factors present postmenopausally.

## Animal Models

A reliable animal model is invaluable in providing optimal flexibility for examining mechanistic, dose-response relationships and comparative efficacy. Ovarian chemoprevention studies of specific molecular targets could be accomplished with greater efficiency if an accepted rodent model of ovarian cancer were available. Under current circumstances, the opportunity to test the many hypotheses being generated by a growing list of potential agents with activity against targets implicated by the new molecular technologies is limited. Research to evaluate several candidate rodent models is being supported by the National Cancer Institute and its Mouse Consortium, and there has been investigation of both syngeneic models (31) by injecting malignant cells or by transfecting epithelial cells with oncogenes (32) to simulate development of a tumorigenic phenotype with variable success. In the meantime, the most widely accepted animal model for ovarian cancer prevention is the domestic white Leghorn chicken (33). Although investigators at The University of Texas M. D. Anderson Cancer Center (Houston, TX) are performing primate studies because of their close similarity to humans, the absence of tumor formation is a drawback, and little is known about primate ovarian carcinogenesis. The chicken, however, has an extremely high rate of Müllerian cancer, of which 30–50% are oviductal in origin. The spontaneous rate of ovarian cancers was approximately 19% in hens ranging from age 2–7 years (33). The potential utility of immunohistochemical markers in the chicken has been investigated to further develop the chicken (*Gallus domesticus*) as a model for spontaneous ovarian carcinoma. Antibodies used to characterize human tumors that were cross-reactive in chicken carcinomas included cytokeratin AE1/AE3, pan-cytokeratin, EGFR, HER-2, Lewis Y, carcinoembryonic antigen, TAG 72, proliferating cell nuclear antigen, p27, and TGF- $\alpha$  (34). Antibodies that were not cross-reactive included CA125, Ki67, Muc-1, and Muc-2. Oviductal cancers were not differentiated from ovarian cancers, which could limit the applicability of these markers. However, the white leghorn chicken model has considerable merit and is being investigated as a model for chemoprevention with the University of Illinois collaborating with Duke University.

Advantages of the chicken model include a high incidence of epithelial cancers. Disadvantages include a high incidence of oviductal cancers and lower correlation with humans than in primates. A study of progesterin and 4-HPR in the chicken model is currently in progress. It will be interesting to see if marker modulation in this system corresponds to observations in humans and in cynomolgus macaques.

**TKIs.** Although the role of the EGFR family in ovarian carcinogenesis is not fully documented, it has been appreciated for some time that growth regulation and differentiation, in response to these receptors in normal ovarian surface epithelium, follow a complex system of interactions that are tissue specific. EGFR, along with the other three members of this receptor family (HER-2, HER-3, and HER-4), is detected immunohistochemically in normal OSE cells at low or moderate intensity (35, 36). Under normal conditions, epidermal growth factor, TGF- $\alpha$ , and amphiregulin provide growth-stimulatory

signals to EGFR, and growth inhibition is mediated by TGF- $\beta$  autocrine feedback (36).

Expression of the various EGFRs in ovarian cancer has been studied extensively by immunohistochemistry. Abnormal expression implying abnormal signaling through EGFR pathways is a common finding in ovarian malignancy. In contrast to the levels observed in normal ovarian surface epithelium, EGFR may be overexpressed in 50–70% of ovarian cancers (37–40). HER-2 is intensely expressed in approximately 10–20% of ovarian cancers and moderately expressed in another 20–40% more cases (41). Because of heterodimer formation among EGFR family members, multiplex expression of certain receptors may be of particular biological relevance. For instance, coexpression of EGFR and HER-2 has been reported in 30–50% of some case series (38, 39). Although intense expression of EGFR and HER-2 is not seen in normal cells and appears to confer a negative prognosis in malignancy, conflicting results regarding prognostic implications are found in the literature. This confusion should resolve as the understanding of EGFR dysregulation in ovarian cancer is updated. For instance, EGFR overexpression is reported to be associated with serous histology (40), and amphiregulin expression is reported to be associated with mucinous histology (42). These observations suggest that histological heterogeneity of ovarian cancer may contribute to the complexity of interpreting the results of growth factor pathway analysis.

A model of the expression patterns underlying EGFR dysregulation has been developed on the basis of EGFR-mediated signals in human ovarian cancer cell lines (43). In brief, the major observation is that coexpression of EGFR and HER-2 facilitates transactivation by epidermal growth factor and produces a strong mitogenic signal. Coexpression of *Her-2* gene product and EGFR was present in 68% of ovarian cancers (44). Two other observations include: (a) heregulin activates HER-4, either on its own or with HER-3; and (b) HER-3 and HER-4 do not cross-react with EGFR and HER-2 after stimulation with heregulin. Activation of the EGFR and HER-2 heterodimer may be the predominant growth-stimulatory signal in ovarian epithelium, as suggested by the commonly observed overexpression of these two receptors in ovarian cancer (45). If unattenuated, it is plausible that this signal could be a critical driving force in ovarian carcinogenesis. This hypothesis is the basis for testing the potential usefulness of TKIs for preventing the abnormal proliferation of ovarian epithelium that is postulated to precede malignant transformation (see "New Agents").

**COX-2 Expression.** Abnormal COX-2 expression is found in ovarian cancer. Matsumoto *et al.* (46) have reported a series of 28 ovarian carcinomas, of which 79% were positive for COX-2 expression overall, with 61% strongly positive and 18% weakly positive. In contrast, COX-2 expression was not found in the surface epithelium or inclusion cysts of the uninvolved ovaries in this series (46). In these samples, COX-2 expression was correlated with vascular endothelial growth factor expression. Ovarian cancer cell lines that express COX-2 include SKOV3, CAOV3, and OVCAR3, all of which undergo growth suppression by a COX-2 inhibitor *in vitro* (47).

**PI3K.** PI3K represents a node in a pathway downstream to the proliferation signals originating from the EGFR family. The potential importance of PI3K in the malignant process is

suggested by its position in signal transduction between EGFR and AKT2, which has been identified as another site of oncogene abnormality in ovarian cancer (48). PI3K has become a focus of attention because amplification of PI3K activity is observed in approximately 40% of ovarian tumors confined to the subset of tumors with serous histology (49, 50). PTEN mutations predominate in endometrioid ovarian cancers. In addition to increased proliferation, decreased apoptosis is also found in association with increased PI3K activity (51). Amplification of PI3K activity is observed in several ovarian cancer cell lines, including OVCAR3. Mechanistic studies have shown that enzyme activity of PI3K is reduced more than 80% by specific inhibitor LY294002 (52). LY294002 causes a dose-dependent reduction in growth of OVCAR3 cells in culture and reduces tumor burden in nude mice inoculated with OVCAR3 (52).

### Agents

Well-known agents are being considered for use in reducing the risk of developing ovarian cancer. One of these is the OCP. The OCP reduces the risk of developing ovarian cancer with odds ratios ranging from 0.25 to 0.8 (12–14, 53–55). Its protective effect is independent of study design (case-control or cohort study) and study population (population based or hospital based). The reduction in risk appears to persist for up to 10 or more years after discontinuation of OCP. Based on epidemiological findings and assuming a lifetime protective effect and similar protection by all formulations of OCP, it is estimated that more than half of all ovarian cancers in the United States could be prevented by the use of OCP for at least 4 years (53, 54). A recent case control study (56) that compared high-dose OCP with mid-dose OCP showed greater protection from the high-dose OCP. Both progestin and estrogen concentrations are higher in the high-dose pill, but the greater protective effect is attributed to the progestin component. There have not been studies comparing the mid-dose pill with the low-dose pill, but it would be important to determine the effectiveness of the low-dose pill. In monkey studies using both progestins and OCP (57), the progestin arm had more apoptosis of the OSE cells than the OCP arm, suggesting that progestin may be the active component in the OCP, although variances were large, and median values were used because of the large variability. A reanalysis of the data from the cancer and steroid hormone study (58), which examined the strength of OCP components taken by 390 women diagnosed with ovarian cancer compared with controls, found the greatest reduction in ovarian cancer risk associated with the highest progestin potency. In addition to this information, a recent report has explored the relationship between progestin, TGF- $\beta$  expression, and apoptosis in the ovarian epithelium of cynomolgus macaques. Exposure to progestin changed the expression of TGF- $\beta$ , lowering TGF $\beta$ -1 with a corresponding increase in TGF- $\beta$ 2/3, correlating closely with induction of substantial apoptosis in the ovarian epithelium (59). Consequently, the progesterone receptor is a prime candidate as a preventive target. Definitive mechanistic and clinically based studies are of great importance in refining the opportunity to specify a role for progestins, agent/dose/schedule, in ovarian cancer risk management. This body of evidence makes the OCP

(and potentially the progestin component) an excellent candidate chemopreventive agent for ovarian cancer, although more needs to be learned about the mechanism of its protective effect.

Use of the OCP for 5 years decreases the risk of ovarian cancer by 50% (53–55) but reduces the number of ovulatory cycles by approximately 15 percent. Consequently, there is not a linear correlation between the duration of OCP use and the impact on ovarian oncogenesis, suggesting that more complex mechanisms other than just ovulation suppression may be at work in the chemopreventive activity of OCP. Inhibition of gonadotropin release from the pituitary, one of the effects of OCP use, or other unknown effects of estrogens and progestins may also play a role in this chemopreventive activity.

**Retinoids.** As with OCP, the antineoplastic effect of 4-HPR is not completely understood. In the laboratory, the activity of retinoids in ovarian cancer has been studied in various ways. In four different ovarian cancer cell lines, 4-HPR was the most effective at suppressing growth compared with all-*trans*-RA, 9-*cis*-RA, and 13-*cis*-RA (60). Studied in more detail in cell line OVCAR3, 4-HPR was found to weakly activate only RAR $\gamma$ , with induction of apoptosis appearing to occur independent of retinoid receptors. This result is consistent with the classification of 4-HPR as a receptor-independent apoptotic retinoid. In addition to retinoid receptors, several other targets have been suggested to mediate their effect. They include the destruction of the mitochondrial membrane by reactive oxygen species and stabilization of the Rb2/p120 protein that mediates retinoid induced growth arrest (61).

Epidemiological and laboratory data suggest that retinoids may have a role as preventive or therapeutic agents for ovarian cancer (58, 62, 63). Fenretinide or 4-HPR has few side effects compared with other vitamin A derivatives and is currently being used in chemoprevention studies of other organ sites, including lung, head and neck, cervix, and bladder. Experimental studies have demonstrated that retinoids can affect human ovarian cancer cell growth by inhibiting proliferation and inducing apoptosis (58, 62); preliminary data from our laboratory show that 4-HPR induces apoptosis in immortalized OSE cells and in normal OSE cells (64). Some cells respond to 2  $\mu$ M/ml 4-HPR, which might be achievable by oral administration, but others require as high as 10  $\mu$ M/ml to have an effect. The 10  $\mu$ M/ml dose would not be achievable by an oral dose because of side effects, particularly skin and ophthalmic effects (nyctagla). Because epithelial ovarian cancer is thought to arise from a neoplastic process that results from a series of mutations in the OSE cells, the probability of developing a neoplasm would decrease if premalignant or genetically altered cells were eliminated by apoptosis.

In Italy, a randomized trial for the prevention of breast cancer has provided preliminary evidence that retinoids, specifically 4-HPR, may prevent or delay the development of ovarian cancer (63). After surgical treatment for breast cancer, 2972 patients were assigned to treatment with 4-HPR (1422) or placebo (1427) to prevent development of new primary breast cancers. After a median follow-up of 51.9 months, no overall difference in the development of new primary breast cancers was evident; however, there was a significant difference in the numbers of ovarian cancers that developed. During the treatment period, six new cases of ovarian cancer were diagnosed in

the placebo group *versus* none in the 4-HPR group ( $P = 0.0162$ ). After cessation of treatment, there were four additional cancers in the control group and six in the 4-HPR group, suggesting that the effect was not durable. There was also a difference in the characteristics between groups: the control group was more likely to have a BRCA mutation than the 4-HPR group, which suggests that the difference may be due to the BRCA status rather than drug effect (65). However, when combined with the cell work, there is the suggestion that retinoids may have chemopreventive activity in the ovary but may need to be administered for long periods of time or to have a different dosing developed so that higher doses could be achieved without toxicity.

Some low-toxicity retinoids, known as heteroarotinoids, share the receptor-independent apoptotic profile of 4-HPR and may be candidates for development as chemopreventive agents (61). In addition to this group of retinoids, AHPN/CD437, a conformationally restricted retinoid engineered to bind selectively to RAR $\gamma$ , also shares the apoptotic profile of 4-HPR. In ovarian tumor cell cultures, exposure to AHPN was associated with increased expression of *BAX* and decreased expression of *Bcl-2* (66, 67). Agent development for ovarian risk reduction will benefit from mechanistic studies of apoptotic induction by 4-HPR and functionally similar compounds such as AHPN. Retinoids that suppress both growth and survival of abnormal cells hold more promise as chemopreventive agents. Prioritizing retinoids for ovarian cancer prevention will ultimately depend on the overall interrelationship among antiproliferative, differentiating, and apoptotic properties of these compounds.

Although there are still too little data to definitively guide chemopreventive studies, there is compelling evidence that as many as 50% of ovarian cancers may be prevented with OCP, and possibly even more may be prevented if a combination of the active ingredients (presumably progesterone) and a vitamin A derivative could be combined for use in high-risk women. Likewise, a similar reduction might be obtained if the active component of OCP could be administered to low-risk women, the group that develops 90% of the ovarian cancers.

## Biomarkers

Biomarkers need to be identified that are predictive of drug or micronutrient activity and pharmacodynamics because the modulation and utility of particular biomarkers will depend on the drug-specific mechanism of action and conditions of use for any given agent. At the current time, little is known about effective biomarkers for drug activity in ovarian cancer chemoprevention. Markers of proliferation and apoptosis are key biomarkers in prevention. Apoptosis is hypothesized to be one of the major mechanisms by which cells with genetic alterations are eliminated (68). In a pivotal trial, primates were treated for 2 years with OCP, estrogen, progestin, or placebo (69). After this period, ovaries were removed, and their surface epithelia were assessed for apoptosis. OCP and progestin significantly increased apoptosis in OSE cells from 5% (baseline) to 25%, whereas estrogen had no effect. If OCP increased the rate of apoptosis in epithelial cells from human ovaries, as they have been found to do in primate ovaries, apoptotic elimination of aberrant cells might be one of the mechanisms underlying the

chemopreventive effects of OCP. In the primate, the high rate of apoptosis (25%) also indicates that either a very large proportion of OSE cells had underlying genetic abnormalities or a number of NOE cells underwent apoptosis in response to components of the OCP. This 25% frequency of apoptosis in the primates appears particularly high when it is considered that it represents a "snapshot" in time and that many cells may already have undergone apoptosis and been cleared from the system. One possible explanation may be the use of a Triphasil pill that has the highest concentration of the progestin in the third week. These monkeys were sacrificed in the third week of their OCP use.

In cell culture, different retinoids (including 4-HPR and all-*trans*-RA) inhibit proliferation and induce apoptosis in a number of tumor cell types [including ovarian cancer cell lines (57, 70–74)]. In at least some cell lineages, 4-HPR is more effective than other retinoids at inducing apoptosis (57) and can induce apoptosis in cells resistant to conventional retinoids. However, the mechanism by which 4-HPR and other retinoids induce growth inhibition and apoptosis is unclear. Most, but not all, studies indicate a correlation between RAR $\beta$  expression or the ability to induce its expression and the ability of 4-HPR to induce growth inhibition and apoptosis (70–72). However, in ovarian cancer cells, 4-HPR effect appears to be receptor independent (61). Induction of reactive oxygen species is another proposed mechanism (74) of 4-HPR, but there are no data available on OSE cells or ovarian cancer cells. These biomarkers of retinoid activity warrant further exploration.

**Growth Factor Specific.** TGF- $\beta$  is another potential biomarker of chemoprevention. It can induce growth arrest and apoptosis of ovarian cancer cell lines, as well as ovarian cancer cells isolated directly from patients (73, 74). In several cell types, including ovarian stroma, steroid hormones can increase TGF- $\beta$  production and activation (75–79). Whether TGF- $\beta$ , in concert with retinoids or steroids, induces apoptosis in ovarian epithelial cells *in vitro* or, more importantly, *in vivo* remains to be assessed. A recent histochemical evaluation of the primate ovary from primates receiving either progestin alone or the Triphasil OCP found an increase in expression of TGF- $\beta$ 2, but not TGF- $\beta$ 1, suggesting that TGF- $\beta$ 2 may be modulated by OCP, serving as a marker of progestin activity (59). Thus, TGF- $\beta$  and its receptors definitely merit exploration. Other potential biomarkers include proliferative markers, such as Ki67, which are currently being used to evaluate the antiproliferative effects of progesterone.

Stromal cells may play an important role in epithelial carcinogenesis. Both types of cells contribute to the extracellular matrix (80), and stromal cells may influence some of the premalignant changes that occur in the epithelial cells (81). Epithelial ovarian cells express both cytokeratin and vimentin, suggesting a dual phenotype consistent with a multipotential stem cell origin. These epithelial cells produce both epithelial and mesenchymal components of extracellular matrix in tissue culture, which is consistent with their dual phenotype (80). KGF, a mesenchymal growth factor that mediates epithelial-stromal interaction, has been recently studied as a factor in early carcinogenesis (82). Fresh NOE cells, but not stromal cells, were found to highly express KGF, which subsequently growth-stimulated NOE cells in an autocrine manner (83). HGF and its

receptor, Met, have also been studied. HGF and KGF, as well as Kit ligand, have been found to interact and promote NOE cell growth and growth factor expression, suggesting that these may play a role in the growth stimulation that accompanies the carcinogenic process (84). Wong *et al.* (85) found that NOE cells from women without a family history of ovarian cancer down-regulated the HGF receptor with increasing passage, whereas the NOE cells from women with a family history of ovarian cancer did not, suggesting that HGF may be a growth regulator, particularly in cells destined to develop cancers (85). Telomerase may be an additional marker warranting both study and targeting. These biomarkers need further study to understand their role in modulating growth and to determine which growth modulators might be a target for prevention.

### Optical Spectroscopy

One of the more exciting prospects for an early marker of ovarian cancer is optical spectroscopic signatures. In the last decade, substantial research has led to useful optical methods of diagnosing early cancers (86–92). Fluorescence spectroscopy is being used to detect cancers noninvasively in many organ systems using a probe that can interrogate the tissue. The system utilizes redox potential (ratio), which is calculated by  $FAD/(FAD + NADH)$  and is, in part, a measure of the relative hypoxia of the tissue (93). FAD and NAD(P) are reduced in the citric acid cycle (anaerobic glycolysis) to FADH and NAD(P)H, which are used as coenzymes in the electron transport chain. In tumors, these coenzymes will accumulate in their reduced states (NADH and FADH) and produce a unique fluorescence signature (NADH high, FAD low) as a result of alterations in blood flow, decreases in tissue pH, and abnormalities in mitochondria and in transport of electron carrier molecules into the mitochondria (93, 94), where the electron transport chain functions. In a pilot study (95), patterns of fluorescence called excitation/emission matrices differed between normal ovaries and areas of invasive cancer and thus are promising for early detection of ovarian cancer. As anticipated, redox potentials were 50% lower in the cancer than in the normal ovary with peaks at 350 nm (excitation) and 460 nm (emission), representing both collagen and NADH. Even more exciting, however, was our recent primate study of fluorescence spectroscopy as a marker for drug activity in the ovary (96). Unlike cancer, where redox potentials are reduced, redox potentials were increased in response to both OCP and 4-HPR. Changes in fluorescence signatures were hypothesized to be due to a decrease in NAD(P)H and an increase in FAD in response to the drugs. 4-HPR had the least effect on the fluorescence signature of NAD(P)H and the greatest effect on the area corresponding to FAD, in contrast to OCP, suggesting that each agent has a unique effect on cellular metabolism. These agents also produced an increase in the redox-related potential of the target organ, suggesting that hypoxia was less extensive and that the system was more quiescent. Thus, optical spectroscopic signatures may serve as an early marker of drug activity.

Cell culture data (97) show that retinoids can induce apoptosis or inhibit growth in both normal and immortalized OSE cells. Cell studies measuring fluorescence emission compared with percentage of apoptosis and growth inhibition showed that

both apoptosis and growth inhibition correlated with redox ratio ( $P = 0.0274$ ), FAD fluorescence intensity correlated with apoptosis ( $P < 0.001$ ), and NAD(P)H fluorescence intensity correlated with cell survival ( $P = 0.04$ ; Ref. 68).

### New Agents

Given the preponderance of evidence that OCP and/or retinoids have a role to play in ovarian cancer prevention, an initial consideration for the development of new agents would be optimizing the effect of these two categories of agents on ovarian cancer risk reduction. In general, recent progress in pharmacological intervention has been achieved by identifying a specific target that is critical to a pathological process. Disruption of a critical pathological signal by eliminating target function can be the key to disease prevention as well as disease control. Evidence reviewed above suggests that OCPs disrupt a critical pathway in early ovarian carcinogenesis in a substantial proportion of women at risk, but a specific target has not been clearly identified. A recent study supports the theory that the progesterone component of OCPs is responsible for reducing ovarian cancer risk.

In addition to the targets suggested by the observation of ovarian cancer risk reduction associated with the use of OCP or 4-HPR, the molecular analysis of malignant ovarian tumors has identified other candidate prevention targets. Selected oncogenes, growth factors, and signal transduction pathways are connected with ovarian carcinogenesis. The focus on targets related to the EGFR family, P13K, and COX2 is guided not only by preclinical studies but also by the presumption that relatively nontoxic oral agents in these categories are either available or about to become available.

**TKIs.** TKIs are of interest to ovarian cancer prevention because of their effect on the activation of EGFRs. In general, the dose-limiting toxicities for TKIs are skin rash and/or diarrhea. In the prevention setting, it may be possible to identify conditions of use that will avoid these toxicities. Alternatively, if preclinical concepts of prevention are demonstrated with compounds like these, it may be possible to identify less toxic versions of these agents that reduce cancer risk. Beyond EGFR-selective agents, there are TKIs in development with a different range of activity. For instance, CI-1033 is a potent inhibitor of kinase activity across the entire EGFR family. Agents selective for HER-2 are also in development.

Examples of TKIs that are of theoretical interest for testing in ovarian cancer prevention studies are ZD1839 (Iressa), OSI774, and PKI-166 (98, 99). As a pyrolopyrimidine, PKI-166 is structurally different, but like ZD1839 and OSI774, targets EGFR. All of these agents have been used in clinical trials and could be studied in model systems to generate preclinical data relevant to ovarian cancer prevention. EGFR, HER-2, and HER-4 all have a tyrosine kinase domain that can be activated when particular EGFR dimer-ligand complexes are formed. As members of a structural class of compounds known as quinazolines, ZD1839 and OSI774 are structurally similar and selectively deactivate the tyrosine kinase of EGFR. These agents might be particularly useful if it could be demonstrated that an EGFR-selective drug deactivates abnormal EGFR/HER-2 heterodimer expression in ovarian epithelium. In breast cancer cells



that overexpress HER-2, blocking transmodulation through EGFR with an EGFR-specific TKI is known to halt growth (100).

**PI3K Inhibition.** Because of the position downstream to the proliferation signals originating from the EGFR family, agents targeting PI3K might have chemopreventive activity on the basis of an antiproliferative effect. PI3K inhibitors have not reached clinical trials, so future use of agents in this class for chemoprevention awaits their further pharmacological development.

**COX-2 Inhibition.** Another target of chemopreventive interest is COX-2 (101). COX-2 is an inducible enzyme of inflammation, mediating the conversion of arachidonic acid to prostaglandins. Attention has been called to the role that inflammation might play in ovarian carcinogenesis (102). It remains to be seen whether COX-2 inhibitors can be used to modulate COX-2 expression in ovarian epithelium and whether such modulation has a role early in carcinogenesis. As with the other agents mentioned in this review, it would be helpful to have a valid animal model of ovarian carcinogenesis to use in the development of preclinical data.

### Clinical Trials

There are currently ongoing trials of both high low- and high-risk women for investigating chemopreventive agents in the ovary. Fox Chase Cancer Center has a trial using 4-HPR in high-risk women undergoing oophorectomy. The University of Texas M. D. Anderson Cancer Center and the University of Arizona have chemoprevention trials in both low- and high-risk women using OCPs and 4-HPR alone and in combination for women undergoing oophorectomy. This is early exploratory work, and findings from these trials will serve as templates for additional trials. Markers that are elucidated as a result of these trials will help determine which are the best biomarkers for drug activity. Following the results of these and other trials that may be starting in the next decade, larger randomized prospective trials will be important to determine the true preventive activity of these and other agents.

### Discussion

Of the four criteria given in the opening definition of IEN, the most difficult to pinpoint in ovarian cancer is the abnormal phenotype. Although access to early ovarian neoplasia is limited by anatomical circumstance, there may, in fact, be biological reasons why an understanding of ovarian IEN is elusive. Ovarian cancer has been described as diseases (37), and, in fact, more than 40 histological entities contribute to the WHO classification of epithelial tumor types. Heterogeneity in ovarian cancer histology suggests a corresponding complexity in IEN. Also, it is recognized that malignant cells with a specific and identifying molecular fingerprint are not always histologically unidentifiable in seemingly normal epithelium adjacent to tumor (34). Given these observations, it may ultimately be necessary to rely on the genetic and consequent functional abnormalities to identify the precursor population of cells that gives rise to invasive ovarian cancer. In addition to the spectroscopic technique described above, genomic and proteomic methods are now being developed that may facilitate the definition of localized precursors

of ovarian cancer (103). The possibility of analyzing proteins characteristic of cancer risk and shed from ovarian IEN into serum (104–107) appears to offer an attractive alternative to the direct assessment of the ovarian epithelium by microlaparotomy for examination by spectroscopy or biopsy and subsequent microdissection.

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